Poster Session 1 – Biopharmaceutics

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Impact of formulation variables on the in vitro release profiles of furosemide gastric floating drug delivery system

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It was proposed to investigate the effect of formulation composition (amount of HPMC, sodium bicarbonate and alginic acid) on in vitro drug release and floating properties of a poorly water-soluble drug (furosemide). Matrix tablets were prepared by direct compression using a 10-station tablet machine (Rimek Mini Press-I, India; Table 1). In vitro dissolution studies were performed in 900 mL of phosphate buffer (pH 5.8, 37 ± 0.5, 100 ± 2 rev min^{-1}) using USP apparatus-I (GMP model, Electrolab, TDT-08L, India). The Korsmeyer & Peppas (Peppas 1985) equation was used to compare the drug release kinetics and mechanism from matrix tablets. In vitro floating properties were observed by placing tablets in 200 mL of 0.1 M HCl and lag time, and the axial and radial swelling of tablets were determined after 8 h of floating study. In vitro dissolution data showed that release characteristics were dependent on the amount of HPMC with drug release decreasing with increasing polymer concentration. As polymer concentration increases there is a corresponding increase in gel layer thickness with a decreased tendency of solvent penetration into the matrix and decreased drug release. Sodium alginate does not appear to offer any delay in drug release when formulated with higher viscosity grade HPMC at the concentrations tested. Sodium bicarbonate had an appreciable effect on in vitro release of furosemide with increasing concentration increasing release rate. As sodium bicarbonate effervesces following contact with the dissolution medium, the resultant pores create additional access channels for dissolution media within the tablet matrix. A correlation between HPMC, sodium bicarbonate and sodium alginate content and release rate ($R^2 = 0.83$–0.954), and Korsmeyer Peppas variables (K and n values). Most of the formulations showed excellent in vitro floating characteristics for more than 7 h with lag times of less than 10 min and appreciable radial (> 50%) and axial (> 100%) swelling after 8 h of study. In conclusion, formulation compositions of matrix tablets had a significant effect on in vitro performance.

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Table 1 Formulation composition and performance of matrix tablets

Formulation					Release analysis	
Code	Fu mg	SA mg	SB mg	HPMC mg	n	K (h^{-n})
F1	40	5	10	60	0.40 ± 0.07	0.14 ± 0.04
F2	40	5	10	70	0.41 ± 0.02	0.25 ± 0.02
F3	40	5	10	80	0.56 ± 0.05	0.19 ± 0.03
F4	40	5	10	90	0.63 ± 0.04	0.15 ± 0.01
F5	40	5	10	100	0.67 ± 0.04	0.13 ± 0.00
F6	40	5	0	70	0.65 ± 0.05	0.06 ± 0.01
F7	40	5	5	70	0.64 ± 0.03	0.12 ± 0.01
F8	40	5	15	70	0.39 ± 0.04	0.39 ± 0.05
F9	40	5	20	70	0.37 ± 0.03	0.45 ± 0.05
F10	40	0	10	70	0.60 ± 0.06	0.24 ± 0.04
F11	40	10	10	70	0.73 ± 0.03	0.15 ± 0.06
F12	40	20	10	70	0.78 ± 0.04	0.13 ± 0.01

Formulation: Fu, furosemide; SA, sodium alginate; SB, sodium bicarbonate; HPMC, HPMC K4 M; Xanthan gum (3 mg). In addition the following excipients were used in all formulations: magnesium stearate (5 mg), purified talc (2 mg), cross-linked PVP (55 mg), lactose fast flow (10 mg).

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039
Ibuprofen dissolution from solid dispersions

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Solid dispersions can be used to improve dissolution of poorly water-soluble drugs such as ibuprofen (Chiou & Riegelman 1971). Polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) are common polymeric carriers in such systems. The mechanisms underpinning the observed improvements in dissolution rate are not fully understood and rely on an understanding of the dissolution behaviour of both components of the solid dispersion. In this study, the dissolution of ibuprofen and polymers (PVP-40 and PEG-8000) were investigated using UV spectroscopy and microviscometry, respectively. The ibuprofen:PVP-40 solid dispersions, at a ratio of 1:2, were prepared using spray drying of an ethanolic solution. The ibuprofen:PEG8000 solid dispersions at a ratio of 1:2 were prepared using the co-melting method. Dissolution was carried out in phosphate buffer (pH 6.8) using a standard USP II dissolution apparatus. Microviscometry was used to study polymer dissolution. It measures small changes in the viscosity of the medium as the polymer dissolves from the solid dispersion. Standard polymer solutions were prepared in phosphate buffer (pH 6.8) and measured using microviscometry (Esnaashari et al 2005). Calibration curves were plotted and were found to be linear over the concentration range in these experiments. The viscosity of the dissolution medium containing ibuprofen was found not to differ from the buffer control. Ibuprofen dissolution was found to be greatly enhanced by the formation of a solid dispersion compared with the pure drug (Table 1). Only 8% of the ibuprofen dissolved at 5 min and 47% at 30 min. From solid dispersions, 30% and 43% of the drug dissolved at 5 min and 100% and 83% dissolved at 60 min from PVP-40 and PEG8000, respectively (Table 1). For PVP, dissolution of ibuprofen has been shown to follow the dissolution of the polymer, therefore, it can be suggested that polymer dissolution governs the drug dissolution from the solid dispersion (Esnaashari et al 2005). However, dissolution of PEG8000 was faster than ibuprofen from the dispersion. The mechanisms underlying this improvement in dissolution are yet to be fully elucidated. Different mechanisms may dominate in different solid dispersion systems and from different drug/polymer ratios. Improved wettability, local solubilisation and particle size reduction have been argued to impart the improvement in dissolution from low molecular weight PVP and from PEG solid dispersions. The use of microviscometry offers an easy technique to study polymer dissolution in drug-release studies. Further work is to be undertaken to characterise the solid dispersions and elucidate the mechanisms governing ibuprofen release from PVP and PEG solid dispersions.

Table 1 Dissolution of ibuprofen (IB) and polymers from solid dispersions

	% Dissolved 5 min	30 min
Pure IB	8	47
IB from PVP-40 solid disp	30	100
IB from PEG8000 solid disp	43	83
PVP from solid disp	42	73
PEG from solid disp	93	99

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Poster Session 1 – Pharmacognosy

040
Essential oil composition and antibacterial activity of *Stachys acerosa* Boiss

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Stachys acerosa Boiss. (Lamiaceae) is an endemic plant of Iran. Other species of *Stachys*, such as *S. lavandulifolia*, have been used for gastrointestinal and female hormonal disorders in Iranian traditional medicine. In this investigation, essential oils composition and antibacterial activity of the flowering and non flowering tops of *S. acerosa* were studied. The plant materials (flowering and non flowering tops) were subjected to hydro distillation using Clevenger-type apparatus. Essential oils were analysed by GC-MS. Identification of compounds was based on a comparison of their mass spectra with standards. Confirmations of compound identities were achieved by their retention indices (Kovats 1958; Adams 2001). Bioautography method (Vanden Berghe & Vlietinck 1991) was used to screen for antibacterial activity on silica gel GF254 TLC plates with toluene–ethyl acetate (93:7) as mobile phase (Wagner & Bladt 1996), on six standard strains: *Staphylococcus aurous, S. epidermidis, E. coli, Klebsiella pneumonia, Bacillus subtilis* and *Pseudomonas aeroginos*. Essential oils yielded 0.10%

and 0.12% and major constituents of the oils were *cis*-chrysanthenyl acetate (36.1%) and linalool (21.6%), respectively. Twenty-nine compounds of the essential oil of the flowering tops were identified and 37 of the 38 compounds in the essential oil of the non flowering tops were identified. Carvacrol, only in the essential oil of non flowering tops with Rf = 0.5 on TLC plates, showed antibacterial activity on all six bacterial strains and was identified by GC-MS after purification on PTLC. Antibacterial activity was also reported from other species of *Stachys*, such as *S. officinalis, S. germanica, S. sylvatica, S. plumose* and *S. reca* (Skaltsa et al 2003) but antibacterial compounds were not identified. Carvacrol was reported as a very potent antibacterial compound. Essential oils of another species of *Stachys* have chrysanthenyl acetate and linalool but not as major components. Germacrene-D in *S. laxa* and *S. sylvatica*, and linalyl acetate in *S. iberica* were the major components of the essential oils.

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Poster Session 1 – Chemistry

041
GRIND-based QSPR method to predict the enantiomeric excess in catalysis

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Quantitative Structure Property/Activity Relationship (QSP/AR) methodology is a chemo-informatic technique successfully applied to a wide range of chemical and biological problems. The generation of a mathematical model, based on previously tested molecules, capable of predicting the behaviour of new compounds can reduce considerable synthetic effort. To study chirality as a property, 3D-descriptors become the obvious choice. Of these, molecular interaction field (MIF) based descriptors have shown to work particularly well in drug design. To date, only a couple of papers have been published to predict the enantiomeric excess of chiral catalysts using the CoMFA analysis. This approach involves the alignment step of all the molecules, which is only straightforward when they all present a substructure in common. We have shown (Morao et al 2005) that GRid INdependent Descriptors (GRIND methodology) give comparable and even better results than ComFA analyses but with no need of the superimposition step. Two different asymmetric reactions are here presented and analysed. The first one consists of a Diels Alder cycloaddition that affords the formation of quinulidine derivates. The second reaction is the reduction of acetophenone with borane. The working procedure can be itemised into five different steps: geometry optimisation of the ligand-metal complex at PM3 semiempirical level; calculation of molecular interaction field of the ligand using standard probes (DRY, N1, O and TIP); filtered points derivation; generation of GRIND descriptors; and application of the diagnostic model (PLS). To carry out the whole process only two user-friendly packages are needed (SPARTAN and ALMOND). The results obtained using this new methodology are summarised in Table 1. GRIND descriptors are capable to fit the experimental data (training set of 18 and 24 ligands for reaction 1 and 2, respectively) given the cross correlation values (r^2) is over 0.9. The internal validation of these QSPR equations using three component random groups also presents a high prediction power (q^2). Finally, the external validation using four new chiral ligands in both reactions showed that the highest residual is less than 15 units. Therefore, we propose this fast and straightforward alignment-independent QSPR methodology for the prediction of the enantiomeric excess in asymmetric catalysis.

Table 1 Statistical information of the QSPR models generated

Reaction	Training set	r^2	q^2
1	18	0.9	0.5
2	24	0.9	0.8

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042
Tight-binding inhibitors as the first active site titrant assays of thymidine phosphorylase from *Escherichia coli*

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Thymidine phosphorylase (TP, EC 2.4.2.4), by catalysing the reversible phosphorolysis of (substituted-)uracil 2'-deoxynucleosides to give 2'-deoxyribose-1-phosphate and (substituted-) uracil, provides both catabolic and salvage routes for pyrimidines. It also provides such metabolic routes for anti-pyrimidine therapeutic agents, such as anti-cancer drugs (e.g. 5-fluorouracil) and antivirals. The 2'-deoxyribose-1-phosphate is rapidly dephosphorylated in the cell to produce 2-D-deoxyribose (an angiogenic factor also known as endothelial cell chemoattractant), which is then exported from the cell (Brown & Bicknell (1998). The discovery that TP is identical with platelet-derived endothelial cell growth factor (PD-ECGF) and that TP has angiogenic activity has given major impetus to the design of strong, specific inhibitors of the human enzyme. TP is attracting attention as a cancer target as it plays a role in tumour angiogenesis (Cole et al 1999), and is expressed at higher levels in the plasma of patients with cancer and in solid tumours relative to normal tissues. The objective of this study was to understand the mode of action of some very strong inhibitors of the *E. coli* enzyme, and to determine if their mode of action differs for *E. coli* and human TP. In the course of our work we were also able to develop a convenient assay to determine the concentration of *E. coli* TP – in fact the first active site titration for it. Spectrophotometric assays for TP involve measurements of UV or visible absorbance differences between the nucleoside (thymidine or 5-nitro-2'deoxyuridine) and the base at pH 7.4. 5-Chloro-6-(2′-imino-pyrrolidin-1′-yl)methyl-uracil hydrochloride (TPI, 1) and its 5-bromo analogue (**2**), as well as 6-(2′-amino-imidazol-1′-yl)methyl-5-bromo-uracil (**3**) and its 5-chloro analogue (**4**) act as strong inhibitors of TP (Reigan et al 2005). However, the measured value of I_{50} (the concentration of inhibitor required to give 50% inhibition under standard conditions) apparently depends on the concentration of enzyme used in the case of TP from *E. coli*. A plot of activity against inhibitor concentration gave straight lines with intercepts on the inhibitor concentration axis proportional to the amount of enzyme added. We elucidated the origins of this effect here by demonstrating that compounds **1**–**4** act as stoichiometric inhibitors of recombinant *E. coli* TP even at enzyme concentrations of 1.3 nM. The compounds present the first active-site titrants of recombinant *E. coli* TP and can be used operationally in this way. The exocyclic guanidino analogues of compounds **1**–**4** show time-dependent behaviour: initial tight binding inhibition (not stoichiometric) increases slowly when enzyme is pre-incubated (over some hours) with the inhibitor at room temperature.

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043
Discovery of the novel prodrug DMU-943, a highly selective anticancer agent

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Although the expression levels of many P450s differ between tumour and corresponding normal tissue, CYP1B1 is the only P450 isoform that is significantly and consistently over-expressed in tumours making it an excellent target for the tumour specific activation of anticancer prodrugs (Rooney et al 2004). We have recently patented a number of compounds based on the chalcone and stilbene structures, which show exciting potential as anticancer prodrugs (Potter et al 2001). A problem with using chalcones and stilbenes therapeutically arises from their high lipophilicity, which results in low water solubility and poor pharmacokinetics. One solution to this problem is to incorporate the functionality that has been shown by a structure activity study to be essential for bioactivation into a nitrogen containing heterocycle. This approach can lead to compounds with increased hydrophilicity and improved physicochemical properties (Wang et al 2002). The identification of selective CYP1B1 activated prodrugs is complicated by the down-regulation of P450 expression in most cultured cells. The cytotoxicity of the compounds synthesised in this study